ABSTRACT OF

FOURTH SEMI-ANNUAL PROGRESS REPORT

Report prepared by: Mary Louise Robbins Date: July 31, 1953

NR: 134-069 Period: Jan. 1-June 30, 1953

CONTRACT: Nonr 291(00)

ANNUAL RATE: $5246

CONTRACTOR: The George Washington University

PRINCIPAL INVESTIGATOR: Mary Louise Robbins, Ph.D.

Research Assistant: Gertrude Marie Sheva, M.S.

Technical Assistant: Richard F. Schmitt, M.S.

TITLE OF PROJECT: An Immunological Investigation of the Herpes Simplex Virus

Objectives: a. To perfect a complement fixation test for diagnosis of herpes simplex infection

b. To investigate the antigenic structure of the herpes simplex virus

RESULTS:

Hyperimmune guinea pig sera against three herpes strains have been tested by cross complement fixation tests. Slight, but possibly significant, differences in titer occur. Human sera tested with these same three and four other strains showed marked differences.

Antibody-adsorption studies are being continued. Hyperimmune guinea pig sera have been adsorbed with crude, highly infective mouse brain and tested for neutralizing antibodies in suckling mice and in eggs. Neutralizing antibodies are completely removed or greatly reduced by this technic. There is no indication of antigenic differences among the three strains used for cross adsorption tests.

Amniotic fluids of infected chick embryos, even after concentration, failed to adsorb antibodies. Sera adsorbed with crude mouse brain become anticomplementary and are therefore not satisfactory for complement fixation tests. Mouse brain suspensions, concentrated and partially purified by "Attaclay" and by protamine sulfate, were also ineffective for use in the complement fixation test.

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METHODS AND RESULTS:

Following the observation that herpes-immune human sera showed quantitative differences toward different strains of herpes virus, when tested by complement fixation, antigenic studies were carried out with hyperimmune guinea pig sera against known herpes strains, particularly LF, Z, and EB. LF and Z hyperimmune sera produce only slight, but possibly significant differences in complement fixation titers when tested against 8 virus strains. EB hyperimmune serum indicates a more definite antigenic difference in complement fixation titers when tested against the same 8 virus strains. The following tabulation shows the results with human and with hyperimmune guinea pig sera. (The results with human sera were reported in the Second Semi-Annual Progress Report, July 31, 1952, and are repeated here for comparison.)

COMPLEMENT FIXATION TITERS OF HERPES IMMUNE SERA
AGAINST VARIOUS HERPES STRAINS
(reciprocals of titers)

SERUM	ANTIGEN (Herpes Strain)							
Human	LF	Z	EB	KE	HF	HT	CF	AG
8	128	8	<4	16	16	8	4	4
9	32	<4	-	4	<4	<4	<4	<4
10	32	4	<4	8	8	4	4	4
11	16	<4	<4	<4	<4	<4	4	<4
12	16	4	<4	4	<4	<4	<4	<4
Guinea pig								
Anti-LF	128	64	128	64	32	32	32	32
Anti-Z	128	256	64	128	64	-	32	128
Anti-EB	4	<4	128	<4	<4	<4	<4	<4

In an attempt to bring out whatever antigenic differences may be present, antibody-adsorption technics have been developed. Adsorption with undiluted and concentrated infected amniotic fluid and with infected chorio-allantoic membranes proved ineffective. Crude mouse-brain suspensions rendered the sera anticomplementary, and are, therefore, unsatisfactory for adsorbing sera to be used for complement fixation. They are reasonably satisfactory, however, for adsorbing sera to be used for neutralization tests in both mice and in chick embryos.

Infected mouse brains were homogenized with an equal volume, by weight, of a 1:4 dilution of hyperimmune serum. The diluent in all cases was 3 parts physiological saline to 1 part nutrient broth, plus ten percent antibiotics. The brain suspensions were then incubated in a 37 C water bath for two hours, and refrigerated overnight. The following day, after centrifugation at approximately 3200 rpm for 10 minutes, the sera were diluted and an equal amount of a 1:10 suspension of infected mouse brain was added. The mixtures were again incubated in a 37 C water bath for two hours and kept under refrigeration until injected the same day into chick embryos and suckling mice.

The chief difficulty is an apparent toxicity in high concentrations of sera adsorbed with normal as well as with infected brain. This should be eliminated by using partially purified brain suspensions.

The results of neutralization tests on sera adsorbed with infected mouse brain are shown in the following table:

NEUTRALIZATION TESTS ON CROSS-ADSORBED HERPES-IMMUNE SERA

Serum	Adsorbing Antigen	Test Antigen: EB	Z	LF
		Reciprocals of Titers		
Anti-EB	None	133	512	380
	Z	23	8	<16
	LF	<47	-	-
Anti-Z	None	273	222	192
	EB	<17	<11	<5
	Z	19	<8	<20
	LF	17	-	-
Anti-LF	None	303	>512	374
	EB	-	-	<66
	Z	45	192	300
	LF	27	-	110

Homologous antibody is, in most cases, greatly reduced, as is heterologous antibody. Obviously, no significant differences in strains appear by this method. In those instances where high titers of antibody remain, corresponding tests showed incomplete adsorption of homologous antibody.

Since the above method could not be used for complement fixation, concentrated and partially purified infected mouse brain suspensions are being used as adsorbing antigen. "Attaclay" and protamine sulfate precipitations were ineffective in purifying the mouse brain suspensions for adsorption. Refinements of these and other methods are being tested.

PLANS FOR FUTURE:

Immediate: a. Continued study of antigenic relationships among herpes strains by cross-complement fixation tests

b. Development of flocculation reaction

c. Perfection of adsorption tests

d. Effect of passage through various laboratory animals on antigenic structure of the virus

Long range: a. Determination of the antigenic structure of herpes simplex

b. Antigenic relationship of herpes virus to other neutotropic and dermotropic viruses